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A continuous countercurrent supercritical fluid deacidification process for phytosterol ester fortification in rice bran oil☆

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Abstract

This study examines the potential of a continuous countercurrent supercritical carbon dioxide fractionation technique for deacidification of crude rice bran oil. A pilot scale packed column was utilized for the experiments. It was shown that fractionation at low pressure, 138 Bar, and high temperature, 80 °C, effectively removed free fatty acids from crude rice bran oil without any oryzanol loss in the extract fraction. Oryzanol content of the raffinate fraction was three times higher than that of the feed material. Phytosterol fatty acid ester content of the raffinate fraction was also increased during the deacidification process, however the enrichment of these moieties was not as high as that found for oryzanol.

Keywords: Continuous countercurrent processing; Deacidification; Fractionation; Phytosterols; Supercritical carbon dioxide

1. Introduction

Plant sterol esters, also referred to as phytosterol esters, were approved as GRAS (Generally Recognized As Safe) by the US Food and Drug Administration (FDA) for use in margarines and spreads in 1999. In September 2000, the FDA also issued an interim rule that allows health-claims labeling of foods containing phytosterol ester (Anonymous, 2001). Consequently enrichment of foods with phytosterol esters is highly desired for consumer acceptance.

A significant portion of the phytosterols naturally present in the vegetable oils is lost in the byproducts during the edible oil refining process (Dunford, 2001). Commercial phytosterol-enriched products are made with phytosterols isolated from such byproducts. Conventional phytosterol isolation and purification methods from oil processing industry byproducts involve several complex and energy intensive unit operations, such as molecular distillation, liquid-liquid extraction and crystallization.

Recently, we have developed a two-step semicontinuous supercritical carbon dioxide ($SC\text{-}CO_2$) fractionation (SFF) process to enrich phytosterol esters in the vegetable oils (Fig. 1). A provisional US patent application has been filed for this process on December 2001. The approach was to increase the phytosterol ester content of vegetable oils during refining rather than isolating them from the byproducts and then adding them back to the oil (Dunford & King, 2000, 2001). Such a processing scheme simplifies the enrichment process and improves the economic feasibility of the production. We were able to increase the total phytosterol ester content of rice bran oil (RBO; Dunford & King, 2001) and corn fiber oil from $<5\%$ to over 19% utilizing the described SFF process.

In general, the economic feasibility of industrial operations is higher for continuous processes when

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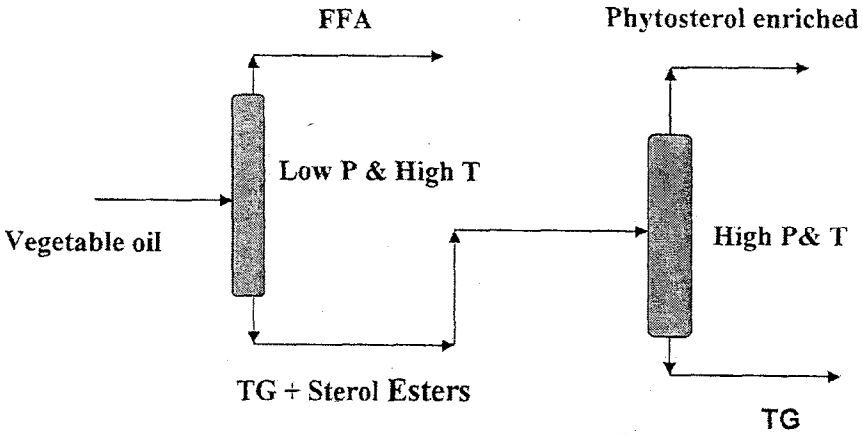

Fig. 1. Schematic diagram of a two-step columnar fractionation process for phytosterol ester enrichment in vegetable oils.

compared to batch or semicontinuous processes. Also countercurrent operations tend to be more efficient due to the larger driving force for mass transfer between solvent and solute. Thus, the objective of this study was to examine the potential of a continuous countercurrent SC-CO_2 fractionation process for enrichment of phytosterols in vegetable oils. The approach was the same as the previous semicontinuous study reported by Dunford and King (2001). In both processes, initially free fatty acids (FFA) would be removed and then a phytosterol-enriched oil fraction would be obtained with a second step extraction process. This particular study focuses on the retention of phytosterol esters in the rice bran oil during the deacidification process while utilizing a continuous countercurrent SFF process.

2. Materials and methods

Crude RBO (Riceland Foods Inc., Stuttgart, AR, USA) was used as feed material for this study. The crude oil was centrifuged at 3000 rpm for 20 min and the resultant precipitate was separated from the oil prior to the SFF experiments. Free fatty acids, free sterols (St), phytosterol fatty acid esters (StE) and oryzanol (FE) contents of the samples were analyzed by HPLC according to Moreau, Powell, and Hicks (1996). The HPLC system consisted of a pump (Model SP 8800, Spectra-Physics, San Jose, CA. USA) an evaporative light-scattering detector (ELSD) (Alltech 500, Alltech Associates, Deerfield, IL, USA), an autosampler (Model SpectraSYSTEM AS 3000, ThermoQuest Inc., San Jose, CA, USA), and a column heater (BioRad Inc., Richmond, CA, USA). ChromQuest software (Version 2.5.1, ThermoQuest Inc.) was utilized for data acquisition and system control.

HPLC-grade solvents, hexane, 2-propanol and acetic acid were purchased from Fisher Scientific (Fairlawn, NJ, USA). Standards of oryzanol (CTC Organics, Atlanta, GA, USA), stigmastanol (TCI, Tokyo Kasei), cholesteryl stearate (Nu-Chek-Prep Inc., Elysian, MN, USA), β-sitosterol and campesterol (Sigma, St. Louis, MO, USA), and α- and β-tocopherol (Aldrich Chemical Co., Milwaukee, WI, USA) were used in this study. Stearic acid (Nu-Chek-Prep Inc., Elysian, MN, USA) was used for FFA quantification.

Lipid and sterol components in the oil samples were separated on a LiChrosorb Diol, 5 μm, 100×3 mm column (Chrompack Inc., Raritan, NJ, USA). The mobile phase gradient consisted of solvent A, hexane/acetic, 1000/1, v/v; and solvent B, acid/2-propanol, 100/1, v/v. The linear gradient timetable was as follows: at 0 min, 100/0; at 5 min 100/0; at 12 min 75/25; at 40 min 75/25; at 41 min 100/0; at 60 min 100/0 (% A and% B respectively). The HPLC eluent flow rate was held constant at 0.5 ml/min. The ELSD detector was operated at 40 °C with nitrogen as the nebulizing gas set at a flow rate of 1.60 l (STP) /min. The column heater temperature was set at 40 °C. Oil samples were dissolved in hexane (about 20 mg/ml) and a 10 μl injection volume was used.

3. SFF column design

An SFF apparatus, designed and installed in-house consisted of a 1.83 m long, 10.2 cm outer diameter stainless steel column (Temco, Inc., Tulsa, OK, USA), was used for the fractionation experiments. A schematic of the SFF apparatus is shown in Fig. 2. The fractionation column consisted of a preheater and four independently controlled temperature zones. The heights of the each heated zone were 43.2, 45.7, 45.7, and 27.9 cm, from top to bottom. Each zone was heated to the desired temperature using 15.2 by 30.5 cm, 0.775 w/cm^2, silicon rubber heaters (Part no. 6012037, Heatcon, Seattle, WA, USA) wrapped around the column and powered through solid state relays (Part no. A2440, Crydom Corporation, San Diego, CA, USA). The temperature of each indivi-

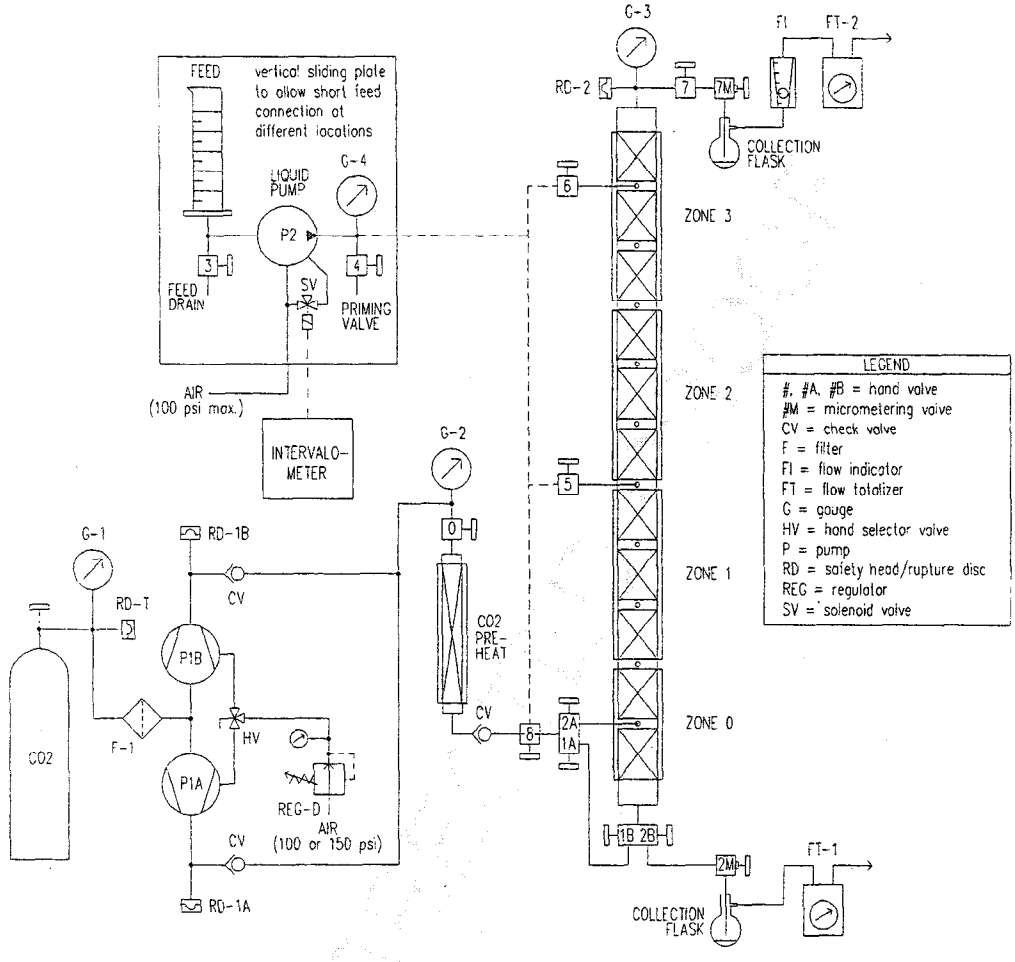

Fig. 2. Schematic diagram of the SFF column design and layout.

dual zone was maintained with PID (Proportional-Integral-Derivative) controllers (Model REX-C4, Syscon-RKC, South Bend, IN, USA) sensing from type-J surface-mount thermocouples (Model CO3, Omega Engineering, Stamford, CT, USA). Internal zone temperatures were monitored with a ten-input digital indicator (Part no.8D45–0080–0603, Watlow, St. Louis, MO, U.S.A.) sensing from seven 1.59 mm diameter type-J thermocouples (Part no. 45–17611, Aminco Superpressure, Silver Spring, MD, USA). Internal zone temperatures remained within 2 °C of the respective set point.

The pilot scale fractionation column's internal dimensions were 1.66 m long and 4.45 cm diameter providing a total column volume of 2.58 l. The column was packed with protruded stainless steel packing material (0.16-inch Pro-Pak, Scientific Development Company, State College, PA, USA) to a density of 0.483 kg/l. Two separate ports at the bottom and side of the lower section of the column allowed for CO_2 input and raffinate removal. The lower section of the column below the CO_2 inlet (the bottom 20.3 cm) was used as a raffinate reservoir.

Carbon dioxide (welding-grade, National Welding Supply, Bloomington, IL, USA) was introduced into the column via an air-driven gas booster pump (Model AGT-62/152, Haskel Inc., Burbank, CA, USA). Feed was metered into the column using an air-driven liquid pump (Model MS-188, Haskel Inc., Burbank, CA, USA) controlled with a pulse-timer (Intervalometer Model 451, GraLab, Dayton, OH, USA). Fluid leaving the column was expanded across a micrometering valve (Part no. 30VRMM4812, Autoclave Engineers, Inc. Erie, PA, USA), thereby permitting the oil fractions to precipitate into a collection vial. The expended gas stream then passed through a dry gas test meter (Model DTM-200A-3, American Meter Company, Philadelphia, PA, USA), allowing measurement of the gas volume used before venting to atmosphere.

4. Column fractionation

Fractionation experiments were carried out in a continuous countercurrent mode of operation. Initially the

column was filled with CO_2 and allowed to equilibrate at the desired temperature and pressure. Then continuous oil and CO_2 flows commenced and extract collection was initiated. Carbon dioxide was allowed to enter the system from the bottom of the column, right above the raffinate collection section. In this particular study, oil was delivered into the system from the top of the column so as to allow countercurrent contact of SC-CO_2 with the feed material. Solute-laden SC-CO_2 then rose upwards in the column and the resultant extract was collected from the top of the column in a collection vial. The oil components, which were not solubilized significantly in SC-CO_2 accumulated in the raffinate reservoir at the bottom of the column. The raffinate reservoir was drained in 15-min intervals to avoid overflow of the raffinate fraction into the fractionation section of the column. During a typical SFF experiment, steady state conditions were reached in the column within the first 3 h of operation. Steady state operation of the column was ascertained by attaining constant weight and composition of the extract fraction collected in 30-min intervals. To evaluate fractionation efficiency, two extract and raffinate samples were collected in 30-min intervals after steady state operation was established in the column. These two samples were analyzed for their lipid and phytosterol content and the averages of the analysis results were reported.

The fractionation experiments were carried out under isobaric and isothermal conditions over the pressure and temperature range of 138–275 Bar and 45–80 °C, respectively. Carbon dioxide and oil flow rates were 2 l/min and 0.7 ml/min, respectively, as measured at ambient conditions. After the completion of the experiment the column was depressurized and residual oil drained off at the end of each run. The column was cleaned between runs at a pressure of 345 Bar and temperature of 90 °C by flowing CO_2 for more than 6 h.

4.1. Statistical analysis

All fractionation runs and analysis of each extract and raffinate sample were carried out in duplicate and in randomized order with the mean values being reported. Analysis of variance (ANOVA) of the results was performed using General Linear Model procedure of SAS (Software Version 8.1. SAS Institute Inc., Cary, NC, USA). Multiple comparison of the various means were carried out by LSD (Least Significant Difference) test at $P=0.05$.

5. Results and discussion

The reported study focused on the first stage of the recently developed two-step SFF technique (Dunford & King, 2001; see Fig. 1). In this case, the effect of temperature and pressure on the phytosterol content of the resultant oil fractions was examined during the removal of FFA from crude RBO utilizing a continuous countercurrent SFF process. Crude RBO oil was used as starting material for the fractionation experiments.

Table 1 shows the composition of the starting material. It should be noted that FFA composition of the crude RBO is higher (~5%, w/w) than that of the other vegetable oils such as soybean and corn oil (~1–2%, w/w) due to the presence of an active lipase in the rice plant. Hence, high phytosterol and FFA content RBO is an excellent model system applicable to this study.

The solute loading of the SC-CO_2 defined as 1 mg of extract collected from 1 g of CO_2, increased with increasing pressure and decreasing temperature as shown in Fig. 3. This can be explained by the higher density of SC-CO_2 at higher pressures and lower temperatures hence higher solvent power of SC-CO_2 under these conditions. Therefore processing at high pressures and low temperatures requires less solvent (SC-CO_2) and reduces the processing time. However, examination of the extract composition showed that the FFA content of the extracts was lowest at the highest pressure and lowest temperature studied (Fig. 4), indicating that SFF fractionation under these conditions is not suitable for efficient FFA removal from the crude oil. This is in part due to the large amount of TG lost in the extract fraction during high pressure and low temperature processing. For example, there is a higher TG content in the extracts at a higher pressure and lower temperature (i.e. 60%, w/w TG at 275 Bar and 45 °C as compared to <10%, w/w TG at 138 Bar and 80 °C) due to the higher SC-CO_2 density and increased volatility of TGs. These results are similar to the data obtained from the semi-continuous process (Dunford & King, 2000, 2001). These results confirm that the deacidification process should be carried out at lower pressures and high temperatures to expedite FFA removal commensurate with lower TG loss in the extract.

A larger amount of StE was removed with the extract at higher pressures (Fig. 5). At lower pressures StE content of the extract (~1%, w/w) was lower than that of the starting material (2.6%, w/w) indicating that these compounds were concentrated in the raffinate

Table 1
Composition of the crude rice bran oil and a typical SFF raffinate fraction obtained at 138 Bar and 80 °C[1]

	Crude RBO (%, w/w)	RBO SFF Raffinate Fraction (%, w/w)
FFA	5±0.6	2.5±0.5
St	0.70±0.05	0.50±0.03
StE	2.6±0.3	2.9±0.4
FE	1.5±0.3	4.9±0.04

[1] FFA, Free fatty acids, St, Free phytosterols, StE, Fatty acid esters of phytosterols, FE, Ferulic acid esters of phytosterols.

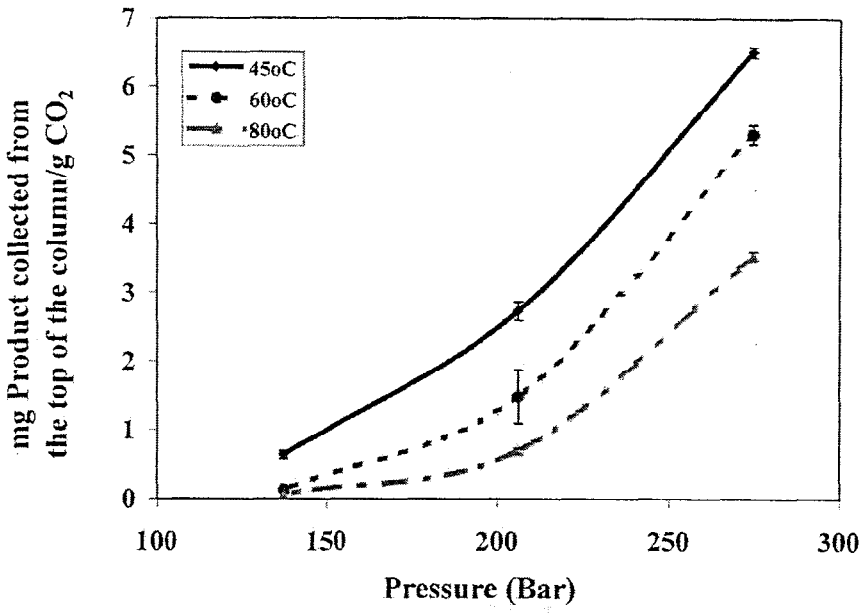

Fig. 3. Effect of temperature and pressure on the SC-CO_2 loading during SFF of RBO.

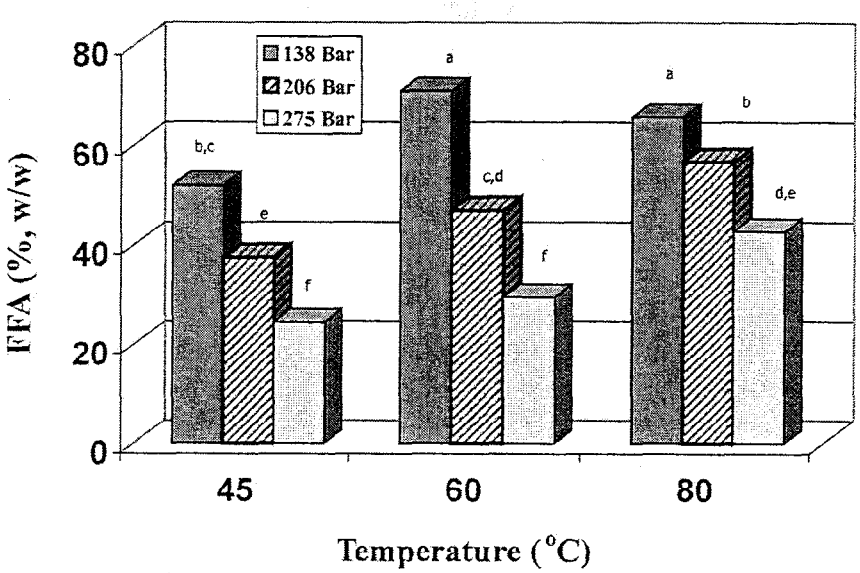

Fig. 4. Effect of temperature and pressure on the FFA composition of SFF extract fractions. Bars with the same letter are not significantly different at $P > 0.05$ level.

fraction. The trend was opposite for the free sterols (Fig. 6). Free sterol content of all the extract fractions was higher (>1%, w/w) than that of the starting material (0.7%, w/w), indicating a significant amount of free sterols loss in the extract. A similar trend was observed for St during the semicontinuous SFF processing (Dunford & King, 2000, 2001).

For this study, oryzanol was detected only in the extracts collected at 275 Bar and 45 °C (2.7%, w/w FE). The oryzanol content of all the other extract samples was lower than the HPLC detection limit. These results demonstrate a significant improvement over the previously reported semicontinuous SFF processing and commercial edible oil deacidification methods. Some oryzanol was lost in all the extracts collected during the semicontinuous SFF process and we were not able to detect any oryzanol in the commercial RBO samples analyzed in our laboratories (Dunford & King, 2001).

Table 1 shows a typical SFF raffinate composition from the continuous countercurrent SC-CO_2 deacidification process. Oryzanol content of the RBO was tripled when removing half of the FFA present in the starting material. Phytosterol fatty acid ester composition of the raffinate fraction was also found to be higher than that of the feed material, however StE enrichment was not as high as that found for oryzanol.

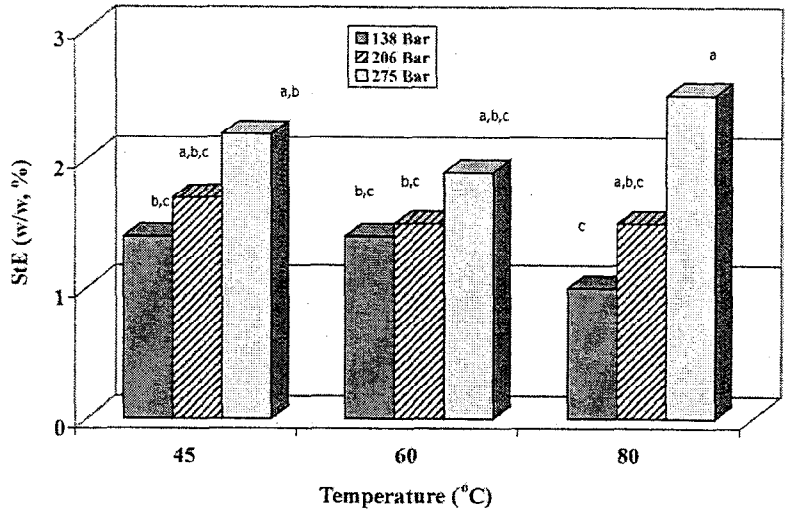

Fig. 5. Effect of temperature and pressure on the StE content of SFF extract fractions. Bars with the same letter are not significantly different at $P > 0.05$ level.

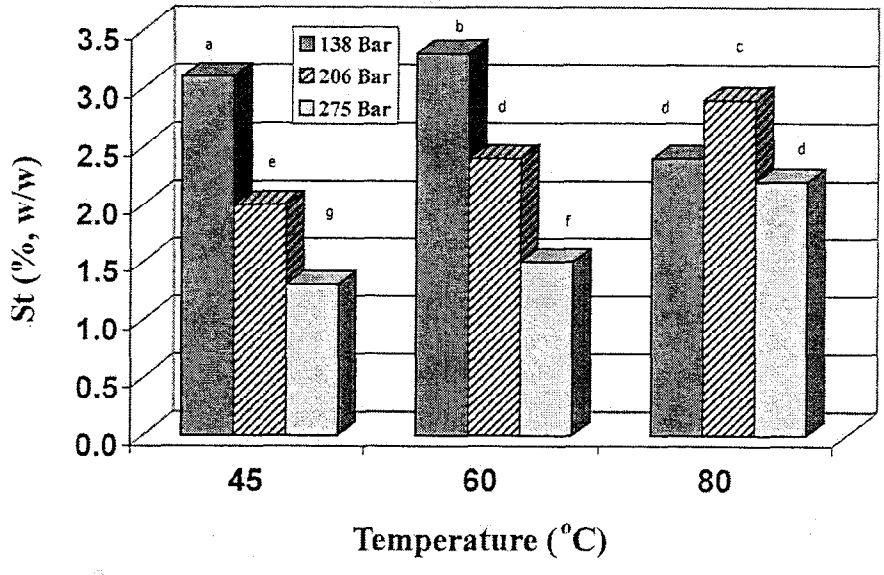

Fig. 6. Effect of temperature and pressure on the St composition of the SFF extract fractions. Bars with the same letter are not significantly different at $P > 0.05$ level.

6. Conclusion

This study demonstrates that a continuous countercurrent SFF column fractionation process carried out at low pressure and high temperature can be utilized to enrich phytosterol esters in the raffinate during the deacidification of crude RBO. The described SFF columnar technique is relatively simple to execute and environmentally benign through the use of SC-CO_2, yielding an end product with no chemical residues.

Uncited reference

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